

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
24 February 2005 (24.02.2005)

PCT

(10) International Publication Number
WO 2005/017522 A3

(51) International Patent Classification?: **G01N 33/15,**
A61K 49/00

(21) International Application Number:
PCT/RO2004/000012

(22) International Filing Date: 20 July 2004 (20.07.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
a 2003 00685 13 August 2003 (13.08.2003) RO

(71) Applicant (for all designated States except US):
INSTITUTUL ONCOLOGIC "PROF. DR. AL. TRESTIOREANU" BUCURESTI [RO/RO]; 252 Sos. Fundeni, Sector 2, R-Bucharest (RO).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **MANOLESCU, Nicolae** [RO/RO]; 82 Tudor Vladimirescu Blvd., bl. 133, entr. 1, apt. 1, Sector 5, R-Bucharest (RO). **BALANESCU, Ion** [RO/RO]; 71 Bulgarus Str., Sector 5, R-Bucharest (RO). **VALECA, Serban, Constantin** [RO/RO]; Calea Craiovei, bl.2, entr. A, apt. 9, R-Pitesti (RO). **TRAICU, Rodin** [RO/RO]; 53 Matei Vasilescu Str., R-Drobeta Tr. Severin (RO). **MARCULESCU, Dumitru** [RO/RO]; Comuna Cerneti, R-Jud. Mehedinti (RO). **NICULITA, Petru** [RO/RO]; 4 Iancu de Hunedoara Blvd., Apt. 7, sector 3, R-Bucharest (RO). **STEFANESCU, Ioan** [RO/RO]; 4 Nicolae Balcescu Blvd., R-Ramnicu Valcea (RO).

TERBEA, Ioan [RO/RO]; 2 Al. Stanila Str., bl. H12, entr. 4, apt. 7, Sector 3, R-Bucharest (RO). **MORARU, Victoria** [RO/RO]; 38 1Decembrie 1918 Blvd., bl. U4, entr. A, apt. 4, R-Bucharest (RO). **COMISEL, Virgiliu** [RO/RO]; 4 Vaporul lui Assan Str., entr. 2, apt. 53, Sector 2, R-Bucharest (RO). **MATEESCU, Corneliu** [RO/RO]; 64 Campia Libertatii Str., bl. 34, entr. C, apt. 115, sector 3, R-Bucharest (RO). **ENCUT, Ioan** [RO/RO]; 26 Sos. Colentina, bl. 64, entr. A, apt. 11, Sector 2, R-Bucharest (RO). **PANAIT, Marieta** [RO/RO]; 18 Sapte Drumuri Str., bl. PM40, entr. A, apt. 16, Sector 3, R-Bucharest (RO). **BEGU, Daniela** [RO/RO]; 17 Barbat Voievod Str., Sector 2, R-Bucharest (RO). **CINCA, Sabin** [RO/RO]; 3 Doamna Ghica Str., bl. 2, entr. 2, apt. 50, Sector 2, R-Bucharest (RO). **GRUIA, Maria-Iuliana** [RO/RO]; 244 Basarabia Blvd., bl. MY8, apt. 19, Sector 3, R-Bucharest (RO). **BALINT, Emilia** [RO/RO]; 1 Marasesti Str., bl. 17, entr. B, apt. 17, Comuna Magurele, R-Jud. Ilfov (RO). **POP, Aneta** [RO/RO]; Lt. Gh. Saidic Str., bl. 24, apt. 54, Sector 6, R-Bucharest (RO).

(74) Agent: **TULUCA, Doina**; Inventa Patent and Trademark Agents, 7 Corneliu Coposu Blvd., bl. 104, entr. 2, floor 1, apt. 31, Sector 3, R-030602 Bucharest (RO).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD,

[Continued on next page]

(54) Title: METHOD FOR IN VIVO DETERMINATION ON TESTED ANIMALS OF THE EFFICIENT CONCENTRATION OF DEUTERIUM DEPLETED WATER FOR CANCER THERAPY

(57) Abstract: As per invention embodiment, the method provides Deuterium Depleted Water administering to tested animals before and after tumoral grafting with animal grafts and it takes the following steps: A) Deuterium Depleted Water administering to Wistar outbred rats by diet, with concentrations of 25 ppm D2, 60 ppm D2, and 100 ppm D2, over a period of 60 days, simultaneously to dieting a control group of animals with water having 150 ppm content of Deuterium (tap water), over the same period of time. B) Viability determination for the tumor cells to be grafted, using *tripan* blue C) Grafting of the animals in the experimental group and the control animals in the 60th day, subcutaneous, with 1×10^7 malign tumor cells in 0.5 cc of normal saline solution of 256 Walker sarcoma (the solid tumor), and T8 Guérin *lymphotropic epithelioma* (the solid tumor) both of them having cells with a viability over 98%. D) Continuously and long-term administering, by diet, of Deuterium Depleted Water, with concentrations of 25 ppm, 60 ppm, 100 ppm Deuterium, period over which the followings are to be done: a. Starting with the 4th post-graft day the tumor nodules measurement and examination is performed on each 2-3 days; b. Monitoring of animals' physiological condition by weekly weighing, monitoring their food and water consumption, notifying the toxic condition occurrence c. After 60 days, when all the animals in control group are dead, preferable between the 160th and 200th day after graft, the effect produced by administering of established concentrations of Deuterium Depleted Water is observed on the surviving animals homeostasis from experimental groups, respectively the way how humoral immune system and cellular immune system of these animals has been influenced, by performing of a series of examination on immunological condition of the animals, namely: leucocytes formula test to establish lymphocytes and blastic cells levels; *hematopoietic* marrow tests to establish the *plasmocytes* and NK-K cells levels. Also, was established the level of the competent immunologic cells in the lymph nodes of the tumoral graft zona. E) Determination of efficient concentration of Deuterium Depleted Water for tested surviving animals depending on new homeostasis occurrence, and on the results obtained related to tumoral regression, as well as on cancer curing cases. This method determines the 60 ppm concentration of Deuterium Depleted Water to be the most efficient for cancer therapy and prophylaxis by continuously and long-term administering of this type of water in daily diet.



MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

- (84) **Designated States** (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- *with international search report*
- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

- (88) **Date of publication of the international search report:**

14 April 2005

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.